PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Y04S012PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/JP2004/016276	International filing date *(day/month/year)* 27.10.2004	Priority date *(day/month/year)* 01.03.2004
International Patent Classification (IPC) or national classification and IPC INV. C12N5/08 C12N5/10		
Applicant KITAKYUSHU FOUNDATION FOR THE ADVANCEMENT ...et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 13 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the report
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27.12.2005	29.05.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/016276

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-3, 5, 8, 15, 16	as originally filed
4, 6, 7, 9-14	received on 27.12.2005 with letter of 23.12.2005

Claims, Numbers

1-20	received on 27.12.2005 with letter of 23.12.2005

Drawings, Sheets

1/3-3/3	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/016276

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7-10,13-16,19,20
	No:	Claims	1-6,11,12,17-18
Inventive step (IS)	Yes:	Claims	7-10,19,20
	No:	Claims	1-6,11-18
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V.

1. The documents mentioned in this report are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

2. The present application relates to a method of providing a human cell line, preferably derived from a myeloma cell line, by selecting strains whose total intracellular protein weight is 0.1-1 mg/10^6 cells, followed by selecting a strain with a doubling time of 18-24 hours and a 90% rate of cloning, followed by mutating said strain with a carcinogen and selecting a mutated cell line with a doubling time of 18-24 hours and a 90% rate of cloning. It further relates to the resulting cell strain, which can be transfected with gene encoding a desired protein for the production of said protein at a yield of 1 ng - 10 µg/day per 10^6 cells. It also relates to a method of producing and purifying proteins using said cell strain and to a pharmaceutical composition comprising the protein produced by said cell strain. It finally relates to two specific cell strains as identified by deposit number, obtained by said method.

3. The human cell strain according to claims 1-6 is defined as a product by process and lacks any technical features that could distinguish the cells from those disclosed in the prior art, such as document D3. The cell line disclosed in D3 also comprises a (spontaneous) mutation, has a doubling time of 19-25 h (see Table 7 on p. 44-45 of D3) and a cloning efficiency of over 90% (see Table 1 on p. 10 of D3). The amount of intracellular protein weight appears to be a parameter which is not disclosed in D3 and can therefore not be used as a distinguishing feature. The rate of protein production disclosed in D3, 0.1 pg-100 ng/cell/day (p. 12, §2 of D3) falls within the range of 1 ng-10 g/day/10^6 cells (claim 7). Furthermore the cell line in D3 can sustain continuous growth in chemically defined (serum free) media at high cell density (p. 10, § 2 and p. 11, § 3) and is used for the production of recombinant proteins by transfecting with a gene encoding said protein. Document D3 also discloses the myeloma cell being derived from human RPMI 8226 cells (p. 8, last §).
Thus D3 anticipates the subject matter of claims 1-6, 11, 12 and 17-18 (Article 33(2) PCT).
Whereas the applicant has argued that the cell line of the present invention is different from known cell lines in that it enables long-term and stable production of

recombinant proteins for longer than 2 months, claims 1-6 are not limited to said feature. The claims refer to 'continuous production' rather than to a clearly defined long term production.
Dependent claims 13-16 do not contain any features which, in combination with the features of any claim to which they refer, could render said claims inventive as such (Article 33(3) PCT).

4. It seems that the specific cell lines as defined in claims 7, 19 and 20 may be different from those disclosed in D3 by possibly having a different mutation. Therefore the objective problem to be solved can be seen as the provision of a further cell line enabling efficient production of recombinant protein. The solution provided are the specific cell lines according to claims 7, 19 and 20, which cell lines enable stable production of recombinant proteins for longer periods, beyond two months or up to one year. None of the prior art documents suggests the possibility that such stable and long term protein production can be achieved. Therefore the subject matter of claims 7, 19 and 20 is considered inventive.

5. With regard to the method according to claims 8-10, it seems that none of the prior art documents suggests the specific steps in the specified order and with the selection criteria (thresholds) as defined in the claims. Therefore said method can be considered inventive.

Re Item VIII.

The cell strain according to claims 1-7 is defined by a result to be achieved (amount of intracellular protein weight, doubling time, cloning efficiency, amount of protein produced over longer time period). Said cell strain can be obtained using the method as defined in claims 8-10. Said method involves mutagenesis with carcinogens, followed by screening. Mutagenesis is considered a random process that relies on chance. The IPEA considers that relying on chance for reproducibility amounts to an undue burden. Therefore the cell strain according to claims 1-7 is considered as insufficiently supported (Article 6 PCT).

document 8); Japanese Patent Laid-Open No. 2003-274963 (Patent document 9); Japanese Patent Laid-Open No. 2002-58476 (Patent document 10); JP-T 2000-506379 (Patent document 11). However, none of these prior art disclosures achieve the abovementioned objective of this invention.

SUMMARY OF THE INVENTION

A novel human cell strain enabling the continuous production of a desired protein with high efficiency, having a novel human cell strain established by transforming a human cell strain whose total intracellular protein weight is 0.1 to 1 mg per 1,000,000 cells; with the novel human cell strain being further characterized in that after a gene encoding a desired protein is transfected into it, the transfected cell is subsequently cultured.

It is embodied in another mode of the invention a novel human cell strain, enabling the continuous production of a desired protein at a yield of 1 ng – 10 μg/day per 1,000,000 cells at least over a 2-month period, having a novel human cell strain established by transforming a specific human cell strain; with the novel human cell strain being further characterized in that after a gene encoding a desired protein is transfected into it, the transfected cell is subsequently cultured in a serum free medium.

It is embodied in a still further mode of the invention a method for selecting a novel human cell strain for producing a desired protein, including the steps of (a) selecting a human cell strain with a total intracellular protein of on or about 0.1 – 1 mg per 1,000,000 cell; and (b) choosing, out of human cell strains with a total intracellular protein of on or about 0.1-1.0mg per 1,000,000 cells, cell clones which have a doubling time of 18 to 24 hours and which have a 90% rate of cloning by limiting dilution method; and mutating the cell clones with carcinogens; and selecting cells out of the mutated cells, which have a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method, to be the novel human cell strain.

BRIEF DESCRIPTION OF THE DRAWINGS

Moreover, in the embodiments of this invention, for producing proteins in mammalian cells, a vector containing a cytomegalovirus promoter, a G418 drug resistant gene, and a gene encoding human antibody heavy chain was constructed, and this vector was transfected with candidates of human cells for producing proteins, then their efficiencies were examined by measure of the expressed proteins. After these steps, the novel human cell strains SC-01MFP and SC-02MFP cells, having a stable and continuous production of the transfected gene-derived proteins with a high efficiency rate of 1 ng – 10 μg/1,000,000cells per day over a incubation period of at least two months or more, preferably over a half-year, and even more preferably over one year, were isolated and established.

The SC-01MFP and SC-02MFP cells pertinent to this embodiment can synthesize and produce proteins of other living species besides proteins of human origin.

The resulting present invention is described in detail with reference to the embodiments and examples, as follows.

The present invention is a method for producing proteins by a human cell stain, and the synthesized proteins depend largely on the characteristics of the human cell strain used. Accordingly, the inventors of this invention acquired a mutated strain, by isolating and selecting a human cell strain from various kinds of human sources, that allows a long term stable protein production, as described below.

The human cell strains prepared for protein production of the invention are four types of human hemocyte cell strains (human leukemiaT-cell strain PEER, human leukemia cell strain SK-729-2, human myeloma cell strain KMS-12BM, RPMI8226) and four types of cancer cell strains (human stomach cancer cell strain TMK-1, human lung cancer cell strain A549, human breast cancer cell strain MCF-7, human lung cancer cell strainPC-8). For the lymphocyte floating cells among these cells, a RPMI1640 medium was used as the basic synthetic medium. Since cancer cells are adherent cells, an ERDF medium was used. As a growth factor, fetal bovine serum (FBS) was used.

First, the total weight of intracellular proteins was set as a criterion for selecting a human cell strain for protein production. It is based on the knowledge of the inventors that larger the total weight of intracellular protein of cells, higher the yield of the protein production when a foreign gene is transfected. Therefore, having a total intracellular protein weight of 0.1 – 1 mg per 1,000,000 cells was set as a criterion, and the human cell strains that meet the criterion were selected.

Next, the proliferation characteristics and cloning efficiencies of the cells were set as selection criteria. It is based on the knowledge of the inventors that the cells that succeed in gene transfection must be multiplied from the state of a single cell at the initial culturing stage, and that they must multiply while resisting physical burdens subjected to the cell due to the gene transfection procedure. In this invention, an extremely high criterion of selecting a cell strain with a cloning rate of over 90% with a doubling time of 18 – 24 hours was applied.

These evaluation and fractionation were performed by cell cloning using limiting dilution and a cell function analysis technique using a flow cytometer.

The variant cells obtained as described were induced to mutate in a medium in which nitrosoguanidine (MNNG), phorbol ester(PMA) and ethylmethane sulfonate(EMS), carcinogenic substances, had been added. Subsequently, clones with high proliferation characteristics were cloned again by limiting dilution, and the ones with a cloning rate of over 90 % were selected under the same conditions used above.

On the other hand, in order to use protein production of the human cell strain selected as described above, as a means to produce medicine and food material, it is difficult to separate/refine only a target protein, since foreign proteins and proteins with unknown components are mixed in the production using a medium containing fetal bovine serum, etc. Moreover, in the case of a highly concentrated culture, an inhibitory growth factor of serum origin may act, thus a serum-free culture is essential. Therefore, in order to enable a serum-free culture for a human cell strain for protein production, such a cell strain was sought for selection. For this type of example, insulin, transferrin, ethanolamine, and sodium selenite were used as growth factors.

explained. However, it is to be understood that they are merely representative examples, thus not limiting the scope of this invention.

(Example 1) Production of human antibody heavy chain protein using the SC-01MFP cell strain

The cell concentration of the SC-01MFP cell strain was prepared to 1x10^7 cells/ml with a phosphate buffered saline (PBS). 500 μl of this cell suspension was added to a sample tube, and 1μl (final concentration of 1μg/ml) of a recombinant gene vector containing a cytomegalovirus promoter, a G418 drug resistant gene, and a gene encoding human antibody heavy chain was added. This [suspension] was transferred to 0.4 cm cuvettes for a Gene Pulsar, transgenic device (using in vivo electroporation). The cuvettes were inserted into the electrode of the Gene Pulsar, the voltage was set at 0.3 kV (0.875kV/cm), then applied at 300 μF.

Next, it was transferred to a RPMI1640 medium, and let stand for 5 minutes in a centrifuge tube. Then this centrifuge tube was centrifuged for 5 minutes at 400Xg. The centrifuged supernatant was discarded, and after being suspended with 5 ml of 15% FBS-RPMI medium, it was dispensed into a 96 well culture plate at 100 μl/well. 2 – 4 days later, GENETICIN; antibiotic G-418 sulfate (final concentration of 2 μg/ml) was added, and selective culturing, where the cells other than the gene-transfected cells were terminated, was carried out.

For several weeks, the medium was changed continuously with fresh medium containing GENETICIN. Since cell proliferation was confirmed in several weeks (2-4 weeks), the protein weight of this antibody heavy chain (γ chain) was measured using an enzyme antibody technique. Table 1 shows this result. After transfecting the gene encoding antibody heavy chain protein into the SC-01MFP cell strain, using the method in this example, starting from the 40th day post transfection for over a one year period, it maintained continuously a production yield of approx. 1-2 μg/ml/107 cells, while being incubated.

Each clone of Table 1, showing the production yield after several days of incubation following the gene transfection, respectively, is an example of representative protein expression. The SC-01MFP cell strain without the gene

transfection did not produce antibody heavy chain proteins. Therefore, it can be concluded that any clones described in this data are expressed by the transfected gene.

Given that at least two months or more, preferably a half year or more, and even more preferably over one year of stable protein production has been sought for a stable industrial level production, the SC-01MFP cell strain of this example meets this criteria, thereby confirming the achievement of one objective of this invention.

Table 1

Productivity of Antibody Heavy Chain Protein using the SC-01MFP cell strain

Clone No.	Productivity (μ g/ml)			
Culture days	40	120	200	360
Clone 1	0.798	1.367	1.952	1.932
Clone 2	0.487	1.321	1.895	1.914

(Example 2) Production of human interleukin 1α (IL-1) protein using the SC−01MFP cell strain

The cell concentration of the SC−01MFP cell strain was prepared to 1×10^7 cells/ml with a saline solution, phosphate buffered saline (PBS). 500 µl of this cell suspension was added to a sample tube, and 1µl (final concentration of 3 µg/ml) of a recombinant gene vector containing a cytomegalovirus promoter, and a gene encoding human IL-1 was added. This [suspension] was transferred to 0.4 cm cuvettes for a Gene Pulsar, transgenic device (using in vivo electroporation). The cuvettes were inserted into the electrode of the gene pulsar, where the voltage was set at 0.3 kV (0.875kV/cm), then applied at 300 µF.

Next, it was transferred to a RPMI1640 medium, and left alone for 5 minutes in a centrifuge tube. Then this centrifuge tube was centrifuged for 5 minutes at 400Xg. The centrifuged supernatant was discarded, and after being suspended with 5 ml of 15% FBS-RPMI medium, it was dispensed into a 96 well culture plate at 100 µl/well. 2 – 4 days later, GENETICIN;antibiotic G-418 sulfate (final concentration of 2 µg/ml)

was added, and selective culturing, where the cells other than the gene-transfected cells were terminated, was carried out.

For several weeks, the medium was changed continuously with fresh medium containing GENETICIN. Since cell proliferation was confirmed in several weeks (2-4 weeks), this IL-1 protein weight was verified using an IL-1 using a specific enzyme antibody technique. Table 2 shows this result. Each clone of Table 2, showing the production yield after over 120 days following the gene transfection, respectively, is an example of representative protein expression. The SC−01MFP cell strains without the gene transfection did not produce human interleukin 1α (IL-1) proteins. Therefore, it can be concluded that any clones described in this data are expressed by the gene transfection.

Also, a composition comprising this human interleukin 1α, a part thereof, and physiologically acceptable carriers is one embodiment of a pharmaceutical composition according to this invention. However, the pharmaceutical composition of this invention is not limited to the one utilizing this human interleukin 1α.

Table 2
Human Interleukin 1α Protein Production Using SC-01MFP Cell Strain

Clone No.	Productivity (μ g/ml)
Clone 1	0.116-0.203
Clone 2	0.145-0.259
Clone 3	0.123-0.285

(Example 3) Production of jellyfish GFP fluorescent dye using the SC−01MFP cell strain

The cell concentration of the SC−01MFP cell strain was prepared to 1X10^7 cells/ml with a phosphate buffered saline (PBS). 500 μl of this cell suspension was added to a sample tube, and 1μl (final concentration of 3 μg/ml) of a recombinant gene vector containing a cytomegalovirus promoter and a gene encoding jellyfish GFP (Green Fluorescent Protein) was added. This suspension was transferred to 0.4

cm cuvettes for a Gene Pulsar, transgenic device (using in-vivo electroporation). The cuvettes were inserted into the electrode of the Gene Pulsar, the voltage was set at 0.3 kV (0.875kV/cm), then applied at 300 μF.

Next, it was transferred to a RPMI1640 medium, and let stand for 5 minutes in a centrifuge tube. Then this centrifuge tube was centrifuged for 5 minutes at 400Xg. The centrifuged supernatant was discarded, and after being suspended with 5 ml of 15% FBS-RPMI medium, it was dispensed into a 96 well culture plate at 100 μl/well. 2 – 4 days later, GENETICIN;antibiotic G-418 sulfate (final concentration of 2 μg/ml) was added, and selective culturing, where the cells other than the gene-transfected cells were terminated, was carried out.

For several weeks, the medium was changed continuously with fresh medium containing GENETICIN. Since cell proliferation was recognized in several weeks (2-4 weeks), the expression of the GFP was measured with excitation of the green fluorescent by epi-illumination inverted microscope to confirm the production of the protein. Figure 1B shows this result. The data of Figure 1A and 1B, showing an image of clones in which the GFP was expressed and for which over 90 days has passed since the gene transfection, is an example of representative clones that expressed proteins.

As shown in the right picture, the GFP expressed cells emit fluorescent light by fluorescence excitation using the fluorescence microscope. The SC–01MFP cell strains without the gene transfection do not produce GFP protein. Therefore, it could be concluded that any clones described in this data are expressed by the gene transfection.

(Example 4) Production of heavy chain protein of human antibody using the SC–02MFP cell strain

The cell concentration of the SC–02MFP cell strain was prepared to 1X10^7 cells/ml with a saline solution, phosphate buffered saline (PBS). 500 μl of this cell suspension was added to a sample tube, and 1μl (final concentration of 1 μg/ml) of a recombinant gene vector containing a cytomegalovirus promoter, a G418 drug

resistance gene, and a gene encoding human antibody heavy chain was added. This [suspension] was transferred to 0.4 cm cuvettes for a Gene Pulsar, transgenic device (using in vivo electroporation). The cuvettes were inserted into the electrode of the Gene Pulsar, voltage was set at 0.4 kV (1.0 kV/cm), then applied at 300 μF.

Next, it was transferred to a RPMI1640 medium, and left alone for 5 minutes in a centrifuge tube. Then this centrifuge tube was centrifuged for 5 minutes at 400Xg. The centrifuged supernatant was discarded, and after being suspended with 5 ml of 15% FBS-RPMI medium, it was dispensed into a 96 well culture plate at 100 μl/well. 2 – 4 days later, GENETICIN;antibiotic G-418 sulfate (final concentration of 2 μg/ml) was added, and selective culturing, where the cells other than the gene-transfected cells were terminated, was carried out.

For several weeks, the medium was changed continuously with fresh medium containing GENETICIN. Since cell proliferation was recognized in several weeks (2-4 weeks), this antibiotic heavy chain (γ chain) protein weight was measured using an enzyme antibody technique. Table 3 shows this result.

Table 3
Productivity of Heavy Chain Protein of Antibody using SC-02MFP Cell Strain

Clone No.	Productivity (μg/ml)			
Culture days	40	80	140	210
Clone 1	1.834	1.057	1.444	0.839
Clone 2	1.803	1.200	1.669	0.671

(Example 5) Serum-free culture of the SC−01MFP cell strain, wherein heavy chain proteins of human antibody have been expressed

The cell concentration of the cell clones, where the SC-01MFP cell strain has been transfected with a gene encoding a human antibody heavy protein and the protein has been expressed for a long-term, is prepared to 1x10^5 cells/ml. Next, it was incubated in a medium ITES-ERDF, where 10 μg/ml of insulin, 20 μg/ml of transferrin, 20 μM of ethanolamine, and 25nM of sodium selenite are added to a

minimal essential medium ERDF (Kyokuto Pharmaceutical) as final concentration, or otherwise incubated only in a minimal essential medium ERDF. As shown in Figure 2A and 2B, cell proliferations of said clone, incubated in only ERDF medium or ITES-ERDF medium, are confirmed as equivalent to or greater than that with the medium ERDR containing fetal bovine serum (FBS), which is ordinarily used for cell cultivation.

(Example 6) Large-scale and high-density culture of the SC–01MFP cell strain, wherein heavy chain proteins of human antibody have been expressed

The cell concentration of the cell clones, where the SC-01MFP cell strain has been transfected with a gene encoding a human antibody heavy protein and the protein has been expressed for a long period of time, is prepared to 0.2×10^7 cells/ml to 1×10^7 cells/ml. Next, it was inoculated to a hollow fiber cartridge (400-011, Spectrum Inc, US) using a medium ITES-ERDF, where either 10% of fetal bovine serum is added, or 10 μg/ml of insulin, 20 μg/ml of transferrin, 20 μM of ethanolamine, and 25nM of sodium selenite are added to a minimal essential medium ERDF (Kyokuto Pharmaceutical) as final concentration; or otherwise only using a minimal essential medium ERDF. After the inoculation, it was incubated using a large-scale and high-density culturing unit (US, Spectrum Inc, CellMax system).

As shown in Figure 3, the SC–01MFP cell strain, expressing heavy chain proteins of human antibody, are mass cultured (10^7 to 10^8 / ml). The concentration of produced protein after this large scale culturing was at a level of 10 μg to 1mg.

According to the compositions described above, the following effects can be obtained.

In other words, the production of conjugated proteins such as glycoprotein that regulate the physiology of the human body, and high-function proteins that possess a number of functions within a molecule, is essential for functional foods and advanced medical fields. Up to now, mainly microorganisms were used; however, with that method it is difficult to produce conjugated proteins. In the cases of using yeasts and advanced cells such as hamster cells, conjugated proteins can be produced. However,

CLAIMS

1. A novel human cell strain enabling the continuous production of a desired protein with high efficiency, comprising:

a novel human cell strain established by transforming a human cell strain whose total intracellular protein weight is 0.1 to 1 mg per 1,000,000 cells;

with said novel human cell strain being further characterized in that after a gene encoding a desired protein is transfected into it, the transfected cell is subsequently cultured.

2. The novel human cell strain of Claim 1, which is established from human myeloma-derived RPMI8226 cells.

3. The novel human cell strain of Claim 1, which is established from human myeloma-derived KMS-12BM cells.

4. The novel human cell strain of Claim 1, wherein said human cell strain is established by choosing, out of human cell strains with a total intracellular protein of on or about 0.1-1.0mg per 1,000,000 cells, cell clones which have a doubling time of 18 to 24 hours and which have a 90% rate of cloning by limiting dilution method; and mutating said cell clones with carcinogens; and selecting cells out of said mutated cells, which have a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method.

5. The novel human cell strain of Claim 4, wherein said carcinogens are selected from the group consisting of nitrosoguanidine (MNNG), phorbol ester (PMA) and ethylmethane sulfonate (EMS).

6. The novel human cell strain of Claim 1,

wherein said novel human cell strain can continuously produce the desired protein with high efficiency by culturing a clone, which has been transfected with a gene encoding the desired protein and has expressed the desired protein, in synthesis minimal essential medium ERDF with or without growth factors.

7. A novel human cell strain, enabling the continuous production of a desired protein at a yield of 1 ng – 10 μg/day per 1,000,000 cells at least over a 2-month period, comprising:

a novel human cell strain established by transforming a specific human cell strain;

with said novel human cell strain being further characterized in that after a gene encoding a desired protein is transfected into it, the transfected cell is subsequently cultured in a serum free medium.

8. A method for selecting a novel human cell strain for producing a desired protein, comprising:

(a) selecting a human cell strain with a total intracellular protein of on or about 0.1 – 1 mg per 1,000,000 cell; and

(b) choosing, out of human cell strains with a total intracellular protein of on or about 0.1-1.0mg per 1,000,000 cells, cell clones which have a doubling time of 18 to 24 hours and which have a 90% rate of cloning by limiting dilution method; and mutating said cell clones with carcinogens; and selecting cells out of said mutated cells, which have a doubling time of 18 to 24 hours and a 90% rate of cloning by limiting dilution method, to be said novel human cell strain.

9. The method for selecting a novel human cell strain of Claim 8, wherein said carcinogens are selected from the group consisting of nitrosoguanidine (MNNG), phorbol ester(PMA) and ethylmethane sulfonate(EMS).

10. The method for selecting a novel human cell strain of Claim 8, wherein said novel human cell strain can be continuously produced with high efficiency by culturing the clone, which has been transfected with the gene encoding the desired protein and has expressed the desired protein, in synthesis minimal essential medium ERDF with or without growth factors.

11. A method for producing proteins, comprising the use of a novel human cell strain as in any of claims 1-7.

12. A method for producing proteins, comprising:

transfecting a gene encoding a desired protein into the novel human cell strain of Claim 1;

and culturing the transfected cells, to continuously produce the desired protein with high efficiency.

13. The method of Claim 12, wherein said transfecting is achieved by employing a vector containing a cytomegalovirus-derived promoter and a gene encoding the desired protein, to produce the desired protein.

14. The method of Claim 12, wherein said novel human cell strain, which has been transfected with a gene encoding a desired protein and which has expressed the desired protein by the clone, is cultured in synthesis minimal essential medium ERDF with or without growth factors.

15. The method for producing protein of Claim 14, wherein said growth factors include insulin, transferrin, ethanolamine, and sodium selenite.

16. The method for producing protein of Claim 14, wherein said novel human cell strain, has been transfected with a gene encoding a desired protein and which has expressed the desired protein by the clone, is cultured in a large-scale and high-density culture (10^7 to 10^8 / ml) with a serum-free medium.

17. A protein purifying method comprising:
using a novel human cell strain as in any of claims 1-7 to produce a protein; and purifying said protein, for which said human cell strain has been transfected with a gene encoding said protein.

18. The purifying method of Claim 17, further comprising producing a highly efficient and highly pure desired protein derived from the gene encoding said protein, by culturing a clone, which has been transfected with a gene encoding the desired protein and which has expressed the desired protein, in synthesis minimal essential medium ERDF with or without growth factors.

19. A novel human cell strain as in any of Claims 1 – 18 wherein said human cell strain is a protein producing cell strain named as SC-01MFP (Accession Number FERM BP-10077).

20. A novel human cell strain as in any of Claims 1 – 18 wherein said human cell strain is a protein producing cell strain named as SC-02MFP (Accession Number FERM BP-10078).